

Fecal Metabolomes in Response to Feed Supplemented with Fermented *Parkia biglobosa* and *Sphenostylis stenocarpa* in Obese Rats

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Abstract

The ubiquitous consumption of junk foods has drastically contributed to the exponential rise in the incidence of obesity. Hence, the present study explores the therapeutic effect of selected indigenous wild bean *Sphenostylis stenocarpa* (*Otili*) and condiment fermented *Parkia biglobosa* (*Iru*) on obese rats. The rats were fed with a high fat diet for four weeks and the gut microbiota was monitored every other day throughout the period of the experiment. Then, the fecal metabolome was analysed by Gas Chromatography Mass Spectroscopy (GC-MS). Although there was a decrease in the mean weight of rats treated with fermented *iru* compared with those given *Otili*, it was not statistically significant ($p \leq 0.05$). The organisms identified from the fecal samples of the fermented *Iru* groups are *Proteus vulgaris*, *Bacillus cereus* and *Escherichia coli* while those identified from the *Otili* group include *Escherichia coli*, and *Citrobacter Freundii*. However, further study revealed that *Otili* and *Iru* had a similar faecal metabolome. Medium chain fatty acids, such as Decanoic acid, Octanoic acid, ethyl tetradecanoate, Hexadecanoic acid, Methyl tetradecanoate, 9-Hexadecanoic acid, Hexadecnoic acid, cis-10-Hepadecanoic acid, are the most common compounds found in this study. This suggests the fact that the associated gut microbiota from breakdown of respective food samples must have actively mediated in their roles of ameliorating the effect of obesity.

Keywords

Microbiota, Metabolomes, MCFA, SCFA, Obese

1. Introduction

Current evidence supports the potential role of the human gut microbiota in obesity. Studies have shown that the bacterial composition of gut microbiota differs between obese and lean individuals; and that a Western-style diet which is high in fat and refined carbohydrates may promote increased intestinal bacteria linked to obesity [1]. This raises the question whether altering the microbiota can modulate the risk of obesity or whether knowledge of an individual's microbiota can be used to develop personalized diets for obesity prevention [2].

The consumption of beans has received increased attention because of the beneficial physiological effects in the prevention and control of broad range of chronic and degenerative diseases such as obesity [3] [4]. Despite this, there is scarce report on African leguminous plants, such as *Parkia biglobosa* (African locust beans) and *Sphenostylis stenocarpa* (African yam bean); locally known as *Iru* and *Otili* in Western Nigeria respectively. The seed of the former is always fermented to produce food condiments for flavouring due to its outstanding protein and amino acid composition [5] [6] [7]. Apart from the nutritional values, fermented African locust bean seeds provide dietary fiber, energy, minerals and vitamins [8] [9]. It also improves sensory properties of foods which include the organoleptic characteristics [10] [11]. However, African yam bean (*Sphenostylis stenocarpa*) Harms is a seed crop, rich in protein, with the potential to contribute to food security [12]. It can be consumed as dry cooked seed or tube. Seeds are usually added to soups, made into sauces, or milled into flour [13] [14]. This yam bean is a very useful crop because of the ability to thrive under extreme conditions, such as high rainfall, acidity and infertile soil and its resistance to several major crop pests [15].

The bacteria in the human gut not only play an important role in digestion, but research indicates that the microbiome could also play a major role in predisposition to obesity [1]. Against this backdrop, this study was aimed at analysing the gut microbiota and faecal metabolomes in response to high fat diet supplemented with fermented *Parkia biglobosa* and *Sphenostylis stenocarpa* in Wistar rats with a view to investigate their anti-obesity potentials.

2. Materials and Methods

2.1. Collection and Preparation of Materials

Dry beans of *Sphenostylis stenocarpa* (*Otili*) were sourced from the bush of Ado Ekiti environment, authenticated by the Chief Botanist of the Department of Plant Science, Ekiti State University and deposited in the University Herbarium (Voucher number-BU-1010065). The bean was sorted; sun dried for some hours

and later blended into a powder form and stored in a tightly sealed container until use.

Unfermented seeds of *Parkia biglobosa* were purchased from a market in Ado-Ekiti, Ekiti State. The seeds were identified and authenticated at the Department of Plant Science, Ekiti State University and deposited in the University Herbarium (Voucher Number UHAE 2020063). The method described by Aderibigbe *et al.* [16] was adopted for the fermentation process. The dried seeds were hand-picked to remove dirt and boiled under pressure for 3 hours. The cooked seeds were dehulled and washed thoroughly to remove the testa. The cotyledons were boiled again for 1 hour. Three hundred grams (300 g) of the boiled substrate were each weighed separately into twenty sterile baking pans. One milliliter (1 ml) of suitably dialyzed starter cultures was used to inoculate each of the baking pans containing the substrate. The inoculated substrate were mixed using flamed spatula and incubated at 35°C for 36 hours.

2.2. Experimental Animals

Following institutional ethical approval (ORD/ETHICS/AD/043); 50 albino rats were obtained from the Animal House, College of Medicine, Ekiti State University Ado Ekiti. They were maintained under standard laboratory conditions and fed rat chow with water *ad libitum*. The rats were grouped into experimental and control groups. They were fed for five weeks with a food formulation of high fat diet and bean as appropriate while the control group was fed with normal rat chow formulated by BioOrganic Feeds (Table 1). Daily food consumption, body weight, behavioral and physiological changes were observed daily for four weeks as shown in Table 2.

Table 1. Animal diet compositions.

Ingredient (g)	Normal diet (ND)	High-fat diet (HFD)
Casein,	200	200
L-Cystine	3	3
Corn starch	285	0
Maltodextrin	35	125
Sucrose	325	80
Cellulose	50	50
Soybean oil	25	35
Lard	20	350
Mineral mix,	10	10
Dicalcium phosphate	13	13
Calcium carbonate	5.5	5.5
Potassium citrate,	16.5	16.5
Vitamin mix,	10	10
Choline bitartrate	2	2
Total	1000	896

Table 2. Experimental design.

s/n	Experimental group	Composition
1	Positive control (Basal)	Normal Chow Diet
2	Fermented group ^a	80 g fermented <i>Iru</i> + 20 g fat
3	<i>Otili</i>	80 g <i>Otili</i> + 20 g fat
4	Fermented group ^b	40 g fermented <i>Iru</i> + 40 g <i>Otili</i> + 20 g fat

2.3. Microbiota Analysis

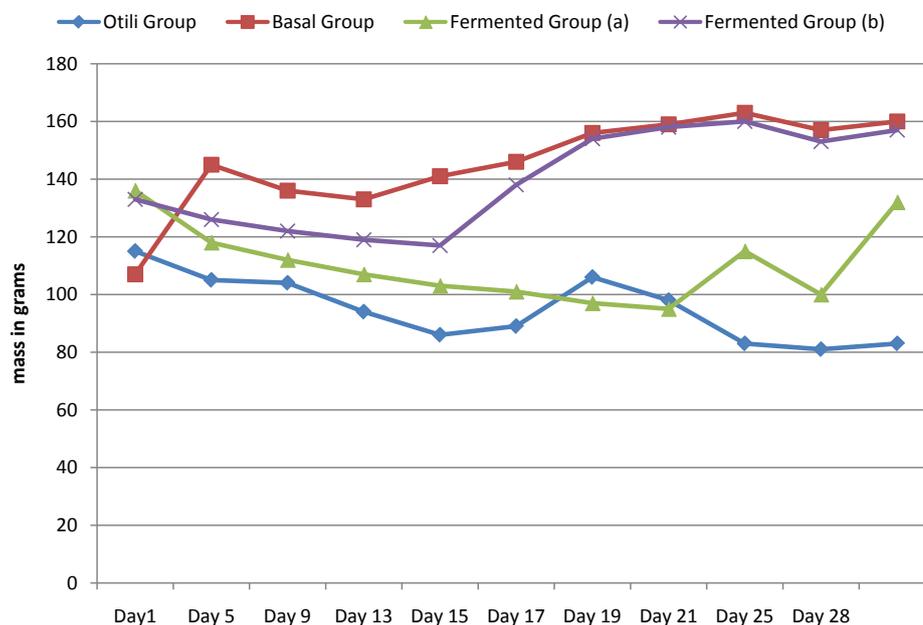
The microbiota analysis was investigated using dilution streak plate method as described by Satish [17]; 1 g of the fecal sample from each group was weighed and kept in sterile test tubes. This was followed by the addition of 10 ml of sterile distilled water and the feces allowed to dissolve. Then, 1 ml of the suspension was pipetted into sterile test tubes containing 9 ml of sterile distilled water and shaken. This was repeated until dilution of 10^1 to 10^{-3} was obtained. Aseptically, already prepared nutrient agar was poured in duplicates into petri dishes and labeled correctly. A loopful from each of dilutions 10^{-3} was streaked on the already prepared nutrient agar and then incubated at a temperature of 37°C for 24 hours. The morphological characteristics and numbers of the colonies was observed and then sub-cultured on new plates containing nutrient agar for pure isolation of microorganisms.

2.4. GC-MS Analysis of Microbiota Products

About 3 - 4 mg fecal sample, was exposed to acid methanolysis in 1 M HCl in methanol at 80°C for 1 hour. The specimen was chromatographically separated on a capillary column with the methylsilicone chemically bonded phase HP-5ms Hewlett-Packard. The comparative concentration of all metabolites was calculated against acetic acid as reference expressing the relative proportion of different metabolites, and the results were expressed in $\mu\text{g/ml}$ [18].

3. Results and Discussion

There has been promising prospects with the manipulation of the gut microbiota to facilitate weight loss or prevent obesity in humans [1]. Possible strategies for obesity prevention and/or treatment include targeting the microbiota, in order to restore or modulate its composition through the consumption of live bacteria (probiotics), nondigestible or limited digestible food constituents such as oligosaccharides (prebiotics), or both (synbiotics), or even fecal transplants [19] [20]. Results from this present study (Figure 1) showed rats fed with *otili* experienced decrease in weight compared to rats mainly fed with normal chow diet. However, combination of fermented *iru*+ *otili*+ high fat diet later caused a decrease in weight perhaps as a result of the *otili* sample present in the food mixture. This finding corroborates report that mice, with a germ-free gut microbiota, are protected against the obesity that develops after consumption of a Western-style,



Duration

Figure 1. Curves showing weight of rats taken every other day.

high fat, sugar-rich diet [21] [22]. It is surmise to say that this present study showed *otili* to be promising in managing some complications associated with obesity such as bodyweight probably due to its influence on the gut microbiota.

Gut microbiome has been identified in the past decade as an important factor involved in obesity, but the magnitude of its contribution to obesity and its related comorbidities is still uncertain.

Olga *et al.* [23] submitted that obesity is closely related to the structure of intestinal micro-biota. Higher amount of Bacteroidetes *i.e.* gram negative bacteria in the gut micro-biota are directly connected with a lean phenotype and with obese individuals who lose weight. Also, reports from both human and animal studies have demonstrated that the relative abundance of Bacteroidetes is reduced by high-fat diet [24]. Although other studies have found changes in gut microbial composition in obese individuals; an increase in the Firmicutes:Bacteroidetes ratio in obesity and an increased abundance of Bacteroidetes during weight loss have not been observed consistently [25] [26]. As shown in **Table 3**, the micro-biota analysis shows morphological characteristics of microorganisms identified. The distinct organisms identified from the fecal samples of the fermented group^a and group^b are *Proteus vulgaris*, *Bacillus cereus* and *Esherichia coli* and those identified from *Otili* are *Escherichia coli* and *Citrobacter Freundii*. These organisms are gut microbes that inhabit many human body sites mostly residing in the gut. The source of the organism *Bacillus spp* in the fermented group is probably due to the fact that *Bacillus subtilis* starter culture was used for the fermentation of the locust bean seeds and hence, the consumption of its microbial cells with the milled seeds by the rats during treatment. *Bacillus subtilis* play

Table 3. Results of microbiota study.

Grp	Nos of Colony	Edge	Color	Shape	Size	Surface	Organism Detected
Positive control	46 ± 0.01	Nil	Cream	Irregular/Round	Large	Smooth	<i>Staphylococcus aureus</i> , <i>Enterobacter aerogenes</i>
Fermented group ^a	32 ± 0.2	Smooth	Pink	Irregular/Round	Large	Smooth	<i>Escherichia coli</i> , <i>Proteus vulgaris</i> <i>Bacillus cereus</i>
Otili group	127 ± 4	Smooth	Cream	Cream	Large	Smooth	<i>Escherichia coli</i> <i>Citrobacter freundii</i>
Fermented group ^b	25 ± 0.01	Smooth	Cream	Irregular/Round	Small	Smooth	<i>Bacillus cereus</i> , <i>Escherichia coli</i> <i>Proteus vulgaris</i>

a significant role in the gut because of their high metabolic activity. Hence, the presence of gram positive *Bacillus cereus* as a part of the gut microbiome may have contributed to the weight loss of the rats. Interestingly, the *Otili* group had distinctive *Escherichia coli* and *Citrobacter freundii*, both gram negative bacteria while the control group fed with normal chow diet had gram positive bacteria- *Staphylococcus aureus*, and *Enterobacter aerogenes*. These corroborate the earlier submission of Evans *et al.* [24] that gram negative bacteria in the gut micro-biota are directly connected with obese individuals who lose weight.

Emerging evidence based on numerous animal studies has shown that the gut microbiota and its metabolites, particularly Small chain fatty acids (SCFAs), play an important role in obesity [27] [28] [29]. There have been conflicting results regarding the relationship between SCFAs and obesity. While some studies have reported a positive correlation between fecal SCFA concentrations and obesity [30] [31] [32], others have reported a negative relationship [19] [33]. In this present study, the control group (Figure 2) showed short lipids in form of 9-Hexadecanoic acid with highest concentration of 40.46 ug/ml. This was followed by Hexadecanoic acid (27.51 ug/ml), Decanoic acid 21.46 ug/ml Octanoic acid (18.65 ug/ml), Dodecanoic acid (17.92 ug/ml), and other unsaturated compounds like Eicosanoic acid as a metabolic product. However, butyrate in form of Butanoic acid, Hexanoic acid and some other compounds were not detected. *Otili* group (Figure 3) was able to produce almost all the compounds and other unsaturated compounds like Heneicosanoic acid; the highest was 9-Hexadecanoic which have the concentration of 99.13 ug/ml followed by Hexadecanoic acid (28.53 ug/ml) but was unable to produce butanoic acid. For Fermented group^a (Figure 4) short lipids in form of 9-Hexadecanoic acid with concentration of 53.83 ug/ml was also found followed by Hexadecanoic acid (26.83 ug/ml), Decanoic acid (21.44 ug/ml), Octanoic acid (18.25 ug/ml) and other unsaturated compounds like Docosanoic acid as metabolic product; however there was no formation of Butyric acid and some other compounds. For Fermented group^b (Figure 5), short lipids in form of Hexadecanoic acid which have the highest concentration of 27.42 ug/ml was found followed by 9-Hexadecanoic acid with the concentration of 18.52 ug/ml but still was unable to produce Butanoic acid. The absence of Butanoic acid (Butyrate) in this present study portends an answer

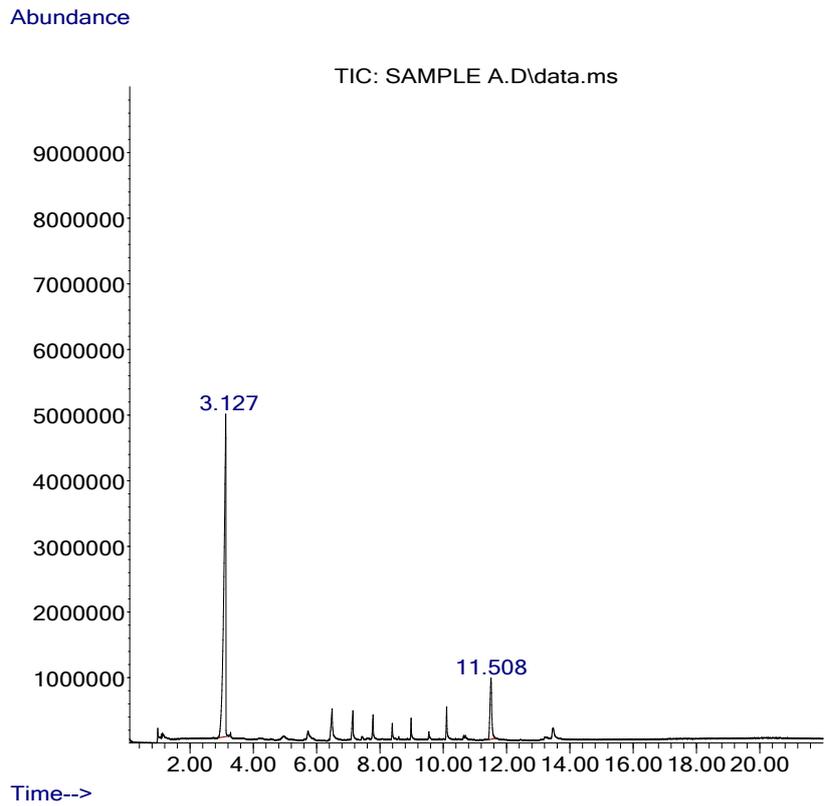


Figure 2. Graph showing the metabolic product from control group.

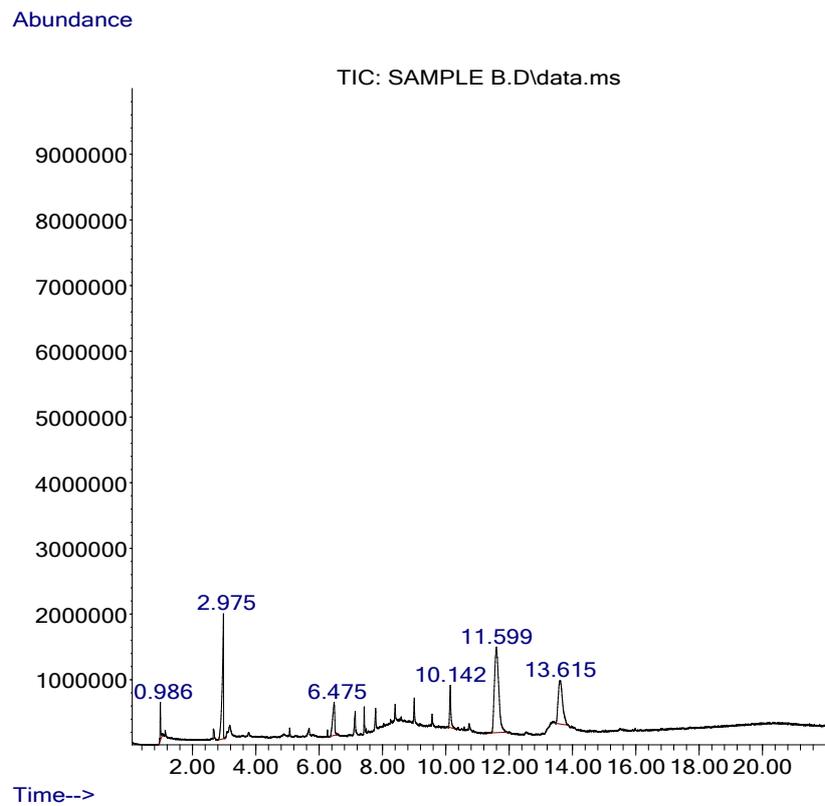


Figure 3. Graph showing the metabolic products from *Otili* group.

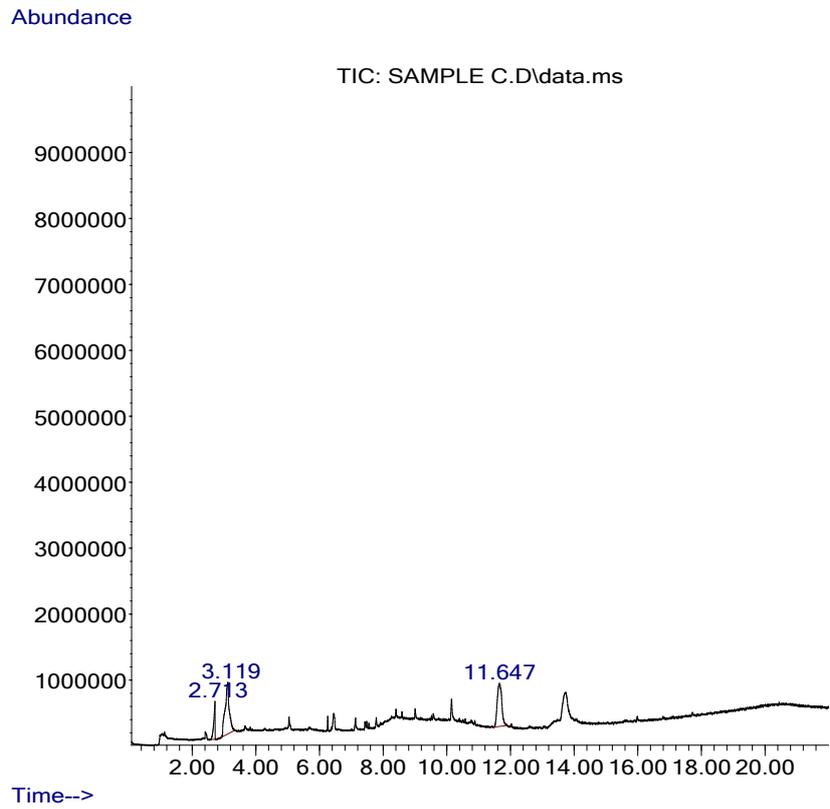


Figure 4. Standard graph showing the metabolic product of fermented group^a.

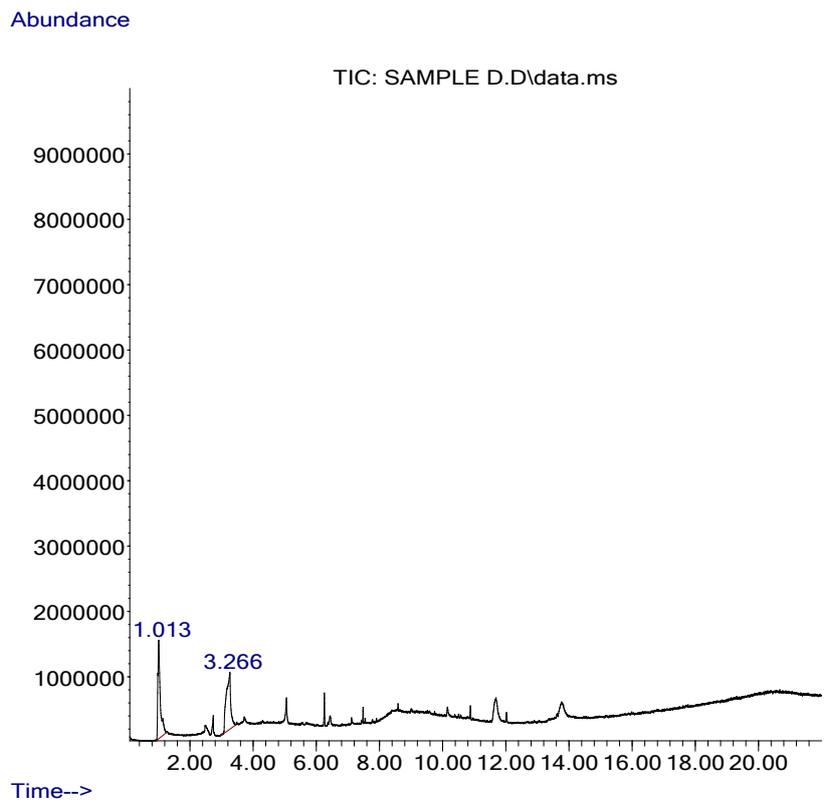


Figure 5. Standard graph showing the metabolic product of fermented group^b.

to the question earlier raised by Lena *et al.* [34] on the link of butyrate with intestinal microbiota and obesity.

4. Conclusion

This study has revealed that *otili* (*Sphenostylis stenocarpa*) and *Iru* (*Parkia biglobosa*) has a similar faecal metabolome. Thus, showing *Iru* and *Otili* are implicated in lipid metabolism suggesting their anti-obesity potential. This demonstrates the increasing benefits of legumes in the diet and offers practical suggestions to aid health care providers in confidently given informed counsel to obese patients on the consumption of these underutilized, but readily available and affordable species of beans, especially in the midst of limited resources in countries such as Nigeria.

Authors' Contributions

The corresponding author, AOA designed and led the study and Author BBC analyzed the data. Author OTR, AMO designed the protocol and prepared the first draft of the manuscript. Authors ADD, AGS, OFC managed the analyses of the study. Authors AOA and OTR handled the literature searches. All authors read and approved the final manuscript.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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